

We claim:

1. A method of treating isolated pancreatic islet cells, comprising:
 - (a) culturing said cells in a medium containing at least one compound selected from the group consisting of an antioxidant, an anti-cytokine, an anti-endotoxin, and an antibiotic; then
 - (b) microencapsulating said cells in a biocompatible microcapsule comprising a hydrogel core and a semipermeable outer membrane, to provide a microcapsule containing living cells therein.
2. A method according to claim 1, wherein said medium contains at least two compounds selected from the group consisting of an antioxidant, an anti-cytokine, an anti-endotoxin, and an antibiotic.
3. A method according to claim 1, wherein said medium contains at least three compounds selected from the group consisting of an antioxidant, an anti-cytokine, an anti-endotoxin, and an antibiotic.
4. A method according to claim 1, wherein said medium contains at least one each of an antioxidant, an anti-cytokine, an anti-endotoxin, and an antibiotic.
5. A method according to claim 1, wherein said antioxidant is selected from the group consisting of glutathione, glutathione monoester, N-acetylcysteine, superoxide dismutase, catalase, vitamin E, α -tocopherol, lipoic acid, lazaroids, butylated hydroxyanisole (BHA), and vitamin K.
6. A method according to claim 1, where said microcapsule comprises a polysaccharide gum surrounded by a semipermeable membrane.
7. A method according to claim 1 where said microcapsule comprises alginate in combination with polylysine, polyornithine, and combinations thereof.

8. A method according to claim 1 wherein said microcapsule has an internal cell-containing core of alginate.

9. A method according to claim 8 wherein said internal cell-containing core of alginate is gelled.

10. A method according to claim 1 wherein said internal cell-containing core of alginate is not gelled.

11. A method according to claim 1 wherein said microcapsule has a diameter of from about 50 μm to about 2 mm.

12. A method according to claim 1 wherein said microcapsule has a diameter of from about 200 μm to about 1000 μm .

13. A method according to claim 1 wherein said microcapsule has a diameter of from about 300 μm to about 700 μm .

14. A method of treating isolated pancreatic islet cells, comprising

(a) cryopreserving said cells in a cryopreservation medium comprising at least one compound selected from the group consisting of an antioxidant, an anti-cytokine, an anti-endotoxin, and an antibiotic; then

(b) thawing said cells, and

(c) encapsulating said cells in a biocompatible microcapsule comprising a hydrogel core and a semipermeable outer membrane, to provide a microcapsule containing living cells therein.

15. A method according to claim 14, wherein said cryopreservation medium contains at least two compounds selected from the group consisting of an antioxidant, an anti-cytokine, an anti-endotoxin, and an antibiotic.

16. A method according to claim 14, wherein said cryopreservation medium contains at least three compounds selected from the group consisting of an antioxidant, an anti-cytokine, an anti-endotoxin, and an antibiotic.

17. A method according to claim 14, wherein said cryopreservation medium contains at least one each of an antioxidant, an anti-cytokine, an anti-endotoxin, and an antibiotic.

18. A method according to claim 14, wherein said antioxidant is selected from the group consisting of glutathione, glutathione monoester, N-acetylcysteine, superoxide dismutase, catalase, vitamin E, α -tocopherol, lipoic acid, lazaroids, butylated hydroxyanisole (BHA), and vitamin K.

19. A method according to claim 14, wherein said anti-endotoxin is selected from the group consisting of L-N^G-Monomethylarginine (L-NMMA), lactoferrin, N-acetylcysteine (NAC), adenosine receptor antagonists and anti-lipopolysaccharide compounds.

20. A method according to claim 14, wherein said anti-cytokine is selected from the group consisting of dimethylthiourea, citiolone, pravastatin sodium, L-N^G-Monomethylarginine (L-NMMA), lactoferrin and 4-methylprednisolone.

21. A method according to claim 14, where said microcapsule comprises a polysaccharide gum surrounded by a semipermeable membrane.

22. A method according to claim 14 where said microcapsule comprises alginate and polylysine.

23. A method according to claim 14 wherein said microcapsule has an internal cell-containing core of alginate.

24. A method according to claim 23 wherein said internal cell-containing core of alginate is gelled.

25. A method according to claim 23 wherein said internal cell-containing core of alginate is not gelled.

26. A method according to claim 14 wherein said microcapsule has a diameter of from about 50 μm to about 2 mm.

27. A method according to claim 14 wherein said microcapsule has a diameter of from about 200 μm to about 1000 μm .

28. A method according to claim 14 wherein said microcapsule has a diameter of from about 300 μm to about 700 μm .

29. A method according to claim 14 wherein, prior to cryopreservation, said islet cells are cultured from about 12 to about 36 hours in the presence of at least one compound selected from the group consisting of an antioxidant, an anti-cytokine, an anti-endotoxin, and an antibiotic.

30. A method of treating biocompatible microcapsules containing mammalian cells, wherein said microcapsule comprises a hydrogel core and a semipermeable outer membrane, comprising:

(a) culturing said microcapsules in a medium comprising at least one compound selected from the group consisting of an antioxidant, an anti-cytokine, an anti-endotoxin, and an antibiotic.

31. A method according to claim 30, wherein said medium contains at least two compounds selected from the group consisting of: antioxidants, anti-cytokines, anti-endotoxins, and antibiotics.

32. A method according to claim 30, wherein said medium contains at least three compounds selected from the group consisting of an antioxidant, an anti-cytokine, an anti-endotoxin, and an antibiotic.

33. A method according to claim 30, wherein said medium contains at least one each of an antioxidant, an anti-cytokine, an anti-endotoxin, and an antibiotic.

34. A method according to claim 30 wherein said mammalian cells are pancreatic islet cells, and wherein glucose-stimulated insulin secretion by said islet cells is enhanced compared to that which would occur in the absence of said culturing step.

35. A method according to claim 30, wherein said antioxidant is selected from the group consisting of glutathione, glutathione monoester, N-acetylcysteine, superoxide dismutase, catalase, vitamin E, α -tocopherol, lipoic acid, lazaroids, butylated hydroxyanisole (BHA), and vitamin K.

36. A method according to claim 30, wherein said anti-endotoxin is selected from the group consisting of L-N^G-Monomethylarginine (L-NMMA), lactoferrin, N-acetylcysteine (NAC), adenosine receptor antagonists and anti-lipopolysaccharide compounds.

37. A method according to claim 30, wherein said anti-cytokine is selected from the group consisting of dimethylthiourea, citiolone, pravastatin sodium, L-N^G-Monomethylarginine (L-NMMA), lactoferrin and 4-methylprednisolone.

38. A method according to claim 30, where said hydrogel core is a polysaccharide gum.

39. A method according to claim 30 where said microcapsule comprises alginate in combination with polylysine, polyornithine, or combinations thereof.

40. A method according to claim 30 wherein said microcapsule has an internal cell-containing core of alginate.

41. A method according to 40 wherein said internal cell-containing core of alginate is gelled.

42. A method according to claim 40 wherein said internal cell-containing core of alginate is not gelled.

43. A method according to claim 30 wherein said microcapsule has a diameter of from about 50 μm to about 2 mm.

44. A method according to claim 30 wherein said microcapsule has a diameter of from about 200 μm to about 1000 μm .

45. A method according to claim 30 wherein said microcapsule has a diameter of from about 300 μm to about 700 μm .

46. A method according to claim 30 wherein, prior to encapsulation, said islet cells are cultured from about 12 to about 36 hours in the presence of at least one compound selected from the group consisting of an antioxidant, an anti-cytokine, an anti-endotoxin, and an antibiotic.

47. A method of preparing microencapsulated pancreatic islet cells comprising:

(a) culturing pancreatic islet cells in a first cell culture medium comprising at least one compound selected from the group consisting of: antioxidants, anti-cytokines, anti-endotoxins, and antibiotics; then

(b) encapsulating pancreatic islet cells in a biocompatible microcapsule comprising a hydrogel core and a semipermeable outer membrane, where said islet cells are present in said core, and

(c) culturing said microcapsule in a second medium comprising at least one compound selected from the group consisting of an antioxidant, an anti-cytokine, an anti-endotoxin, and an antibiotic.

48. A method according to claim 47, wherein said culture media contain at least two compounds selected from the group consisting of an antioxidant, an anti-cytokine, an anti-endotoxin, and an antibiotic.

49. A method according to claim 47, wherein said culture media contains at least three compounds selected from the group consisting of an antioxidant, an anti-cytokine, an anti-endotoxin, and an antibiotic.

50. A method according to claim 47, wherein said culture media contains at least one each of an antioxidant, an anti-cytokine, an anti-endotoxin, and an antibiotic.

51. A method according to claim 47, wherein said antioxidant is selected from the group consisting of glutathione, glutathione monoester, N-acetylcysteine, superoxide dismutase, catalase, vitamin E, α -tocopherol, lipoic acid, lazaroids, butylated hydroxyanisole (BHA), and vitamin K.

52. A method according to claim 47, wherein said anti-endotoxin is selected from the group consisting of L-N^G-Monomethylarginine (L-NMMA), lactoferrin, N-acetylcysteine (NAC), adenosine receptor antagonists and anti-lipopolysaccharide compounds.

53. A method according to claim 47, wherein said anti-cytokine is selected from the group consisting of dimethylthiourea, citiolone, pravastatin sodium, L-N^G-Monomethylarginine (L-NMMA), lactoferrin and 4-methylprednisolone.

54. A method according to claim 47, where said microcapsule core comprises a polysaccharide gum.

55. A method according to claim 47 where said microcapsule comprises alginate in combination with polylysine, polyornithine, or combinations thereof.

56. A method according to claim 47 wherein said microcapsule has an internal cell-containing core of alginate.

57. A method according to 56 wherein said internal cell-containing core of alginate is gelled.

58. A method according to 56 wherein said internal cell-containing core of alginate is not gelled.

59. A method according to claim 47 wherein said microcapsule has a diameter of from about 50 μm to about 2 mm.

60. A method according to claim 47 wherein said microcapsule has a diameter of from about 200 μm to about 1000 μm .

61. A method according to claim 47 wherein said microcapsule has a diameter of from about 300 μm to about 700 μm .

62. A method according to claim 1, wherein said microencapsulating step is followed by the step of incubated said microcapsule containing living cells therein with a physiologically acceptable salt to increase the durability of the microcapsule, while retaining the physiological responsiveness of the living cells contained in the microcapsule.

63. A method according to claim 62, wherein said physiologically acceptable salt is a sulfate salt.

64. A method according to claim 62, wherein said physiologically acceptable salt is selected from the group consisting of sodium sulfate and potassium sulfate.

65. Microencapsulated islet cells produced by a method according to claim 62.

66. Microencapsulated islet cells according to claim 65, said microencapsulated islet cells exhibiting a weight gain of not more than 10 percent by weight over a period of one month in physiological saline solution at 37 degrees Celsius (exhibiting the durability thereof) and exhibiting at least 150 percent basal insulin secretion in response to 16.7 milliMolar glucose challenge in Krebs-Ringer physiological solution at pH 7.4 after said period of one month.

67. A method according to claim 14, wherein said encapsulating step is followed by the step of incubated said microcapsule containing living cells therein with a physiologically acceptable salt to increase the durability of the microcapsule, while retaining the physiological responsiveness of the living cells contained in the microcapsule.

68. A method according to claim 67, wherein said physiologically acceptable salt is a sulfate salt.

69. A method according to claim 67, wherein said physiologically acceptable salt is selected from the group consisting of sodium sulfate and potassium sulfate.

70. Microencapsulated islet cells produced by a method according to claim 67.

71. Microencapsulated islet cells according to claim 70, said microencapsulated islet cells exhibiting a weight gain of not more than 10 percent by weight over a period of one month in physiological saline solution at 37 degrees Celsius (exhibiting the durability thereof) and exhibiting at least 150 percent basal insulin secretion in response to 16.7 milliMolar glucose challenge in Krebs-Ringer physiological solution at pH 7.4 after said period of one month.

72. A method according to claim 47, wherein said encapsulating step is followed by the step of incubated said microcapsule containing living cells therein with a physiologically acceptable salt to increase the durability of the microcapsule, while retaining the physiological responsiveness of the living cells contained in the microcapsule.

73. A method according to claim 72, wherein said physiologically acceptable salt is a sulfate salt.

74. A method according to claim 72, wherein said physiologically acceptable salt is selected from the group consisting of sodium sulfate and potassium sulfate.

75. Microencapsulated islet cells produced by a method according to claim 72.

76. Microencapsulated islet cells according to claim 75, said microencapsulated islet cells exhibiting a weight gain of not more than 10 percent by weight over a period of one month in physiological saline solution at 37 degrees Celsius (exhibiting the durability thereof) and exhibiting at least 150 percent basal insulin secretion in response to 16.7 milliMolar glucose challenge in Krebs-Ringer physiological solution at pH 7.4 after said period of one month.

77. A microencapsulated islet cell product comprising microcapsules containing isolated living pancreatic islet cells therein, said microencapsulated islet cells exhibiting a weight gain of not more than 10 percent by weight over a period of one month in physiological saline solution at 37 degrees Celsius (exhibiting the durability thereof) and exhibiting at least 150 percent basal insulin secretion in response to 16.7 milliMolar glucose challenge in Krebs-Ringer physiological solution at pH 7.4 after said period of one month.

78. A method of isolating pancreatic islet cells, comprising:

(a) digesting pancreatic tissue with a digestion medium, said digestion medium containing an antioxidant, said digesting step carried out for a time sufficient to produce free pancreatic islet cells; and then

(b) collecting said free pancreatic islet cells to produce isolated pancreatic islet cells.

79. A method according to claim 78, said antioxidant included in said digestion medium in an amount sufficient to inhibit reoxygenation injury of said isolated pancreatic islet cells.

80. A method according to claim 78, wherein said antioxidant is selected from the group consisting of vitamin and organic chemical antioxidants.

81. A method according to claim 80, wherein said antioxidant is selected from the group consisting of vitamin C, vitamin E, vitamin K, lipoic acid, lazaroids, and butylated hydroxyanisole.

82. A method according to claim 78, wherein said antioxidant is included in said digestion medium in an amount ranging from 0.1 milliMolar to about 10 milliMolar.

83. A method according to claim 78, wherein said collecting step is followed by the steps of:

(c) culturing said cells in a medium containing at least one compound selected from the group consisting of an antioxidant, an anti-cytokine, an anti-endotoxin, and an antibiotic; then

(d) microencapsulating said cells in a biocompatible microcapsule comprising a hydrogel core and a semipermeable outer membrane, to provide a microcapsule containing living cells therein.